Program/Abstract # 122
The kinome of lung branching morphogenesis — A systems approach to identify phosphoregulators of mouse lung development
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A major goal in understanding organ formation is determining the link between developmental signals and the fundamental molecular pathways responsible for guiding and controlling organogenesis. Although much is known about the molecules that initiate morphogenesis, including Wnts, Hedgehogs, BMPs and growth factors, there is a considerable gap in understanding the downstream targets of these regulators, the links between them, and the mechanisms by which these regulators generate three-dimensional tissues with complex features such as the intricate branching pattern of the lung. In order to fill this gap we have developed a systems approach to obtain a comprehensive understanding of the functional relationship between kinase/phosphatase-regulated molecular pathways and their roles in organizing the formation of the mouse embryonic lung. To this end, we have applied a combination of functional genomics (loss of function screen) with morphological and live imaging analysis in lung explant cultures. Notably, our analysis from the ongoing screen has already revealed a high degree of distinct morphological phenotypes including change in tubule size, branch numbers and position. Moreover, our image analysis does suggest new mechanistic and dynamic properties of epithelial and mesenchymal cells in molding emerging branches during lung development. Thus, the combination of functional genomics and live imaging provides a powerful strategy to further decipher the molecular networks and principles underlying branching morphogenesis.

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Program/Abstract # 123
Cleftin: A novel fibronectin-induced gene that promotes branching morphogenesis
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Branching morphogenesis is a fundamental process shared by many organs. In this process, the epithelium of the mouse submandibular gland forms a cleft that deepens to generate lobules. Cleft formation is an initiation process that is associated with the conversion of cell–cell to cell–matrix adhesions. Fibronectin is known to appear transiently and focally in forming cleft regions, accompanied by an adjacent loss of E-cadherin, but the mechanism is still unknown. Our hypothesis is that cleft epithelia may express functional genes that regulate cleft formation. To test this hypothesis, we identified and characterized novel genes that are expressed in cleft but not bud epithelia by laser microdissection and T7-SAGE libraries. A BTB/POZ domain-containing protein showed differential expression in the cleft epithelium. Expression in developing salivary glands was maximal at embryonic day 13, a period of extensive salivary gland branching. We have termed this protein "cleftin." Highly localized expression of cleftin mRNA was identified around the tip of the cleft by in situ hybridization. To determine whether cleftin can be induced, we tested several matrix molecules. We found that fibronectin can rapidly induce cleftin. Functional analysis of branching morphogenesis using stably transfected MDCK cells showed that cleftin expression decreased E-cadherin. Furthermore, cleftin-expressing cells showed increased branching in 3D collagen gels. Knocking down cleftin using siRNAs inhibited branching in developing mouse salivary glands and lungs. These results indicate that cleftin has an important role in branching morphogenesis.

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Program/Abstract # 124
A novel region in the murine allantois may prevent branching morphogenesis
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The murine allantois is the precursor to the fetal umbilical cord, which channels fetal blood to the chorionic disk for exchange with the mother. During elongation, the distal allantois develops a highly branched vasculature that provides a wide vascular surface area for fusion with the chorion. By contrast, the proximal region develops a thick, unbranched vasculature that amalgamates with the embryonic dorsal aorta and yolk sac blood vessels. Failure of the allantois to properly vascularize and/or fuse with the embryonic and chorion blood vessels has been linked to birth defects such as low birth weight, pre-natal death and cerebral palsy. Here, we tested the hypothesis that the outer covering of the allantois, called the mesothelium, may play a role in vascular branching of the umbilical cord. Our results reveal the presence of a unique feature of the allantoic mesothelium whose properties suggest that it may prevent vascular branching in the proximal allantois whilst facilitating union with embryonic and yolk sac vasculature.

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Program/Abstract # 125
Notch signaling acts at multiple stages to regulate bile duct morphogenesis
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The mammalian biliary system transports bile from the liver to the intestine. Dysfunction of bile ducts is a significant cause of liver disease. The development of bile ducts requires the proper differentiation and morphogenesis of bile-duct precursor cells into a complex three-dimensional structure. Studies of human disease and mouse models have implicated Notch signaling in this process, but the mechanism is still poorly understood. Here, we established a modular transgenic system to heritably activate Notch signaling in hepatoblasts and differentiated liver cells. We find that Notch does not specify bile-duct precursor cell fate during the period of embryonic development. However, Notch acts in a dose-dependent manner to regulate bile duct morphogenesis postnatally. Overexpression of Notch signaling promotes the formation of biliary tubules and the persistence of postnatal bile-duct cells. Taken together, our results suggest that Notch signaling regulates bile duct